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Assessment of bud-grafting and side-grafting techniques to support the improvement of productivity and quality of somatic embryogenesis Cocoa (*Theobroma cacao* L.) in South Sulawesi

Muhammad Basir Nappu, Muh Asaad, Herniwati, Nurlaila, Maintang and Sahardi

Institute for Agricultural Technology, South Sulawesi, Jln. Perintis, Kemerdekaan, Makassar, Indonesia

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Abstract

The results of the previous year study found that the somatic embryogenesis (SE) cocoa plant originated from planting from year 2009 until 2012, has not been in its optimal growth and production. It is reported is a significant mortality rate in the cocoa plant nursery phase or the SE in the vegetative phase. Based on the data and information, it is known that there is a need for the engineering efforts to increase production and quality of SE cocoa through the application of bud grafting and side grafting. The assessment is also expected to support farmer in order to apply the technology of bud grafting and side grafting for SE cocoa plant. The purpose of this study are : a) to find out the improvement of crop productivity and quality of SE cocoa through bud grafting and side grafting, and b) to analyze the response of farmers to increase crop productivity and quality SE cocoa through bud grafting and side grafting. This study was conducted in Bone, and Luwu regency, South Sulawesi from January 2015 to December 2016. This study used a Randomized Block Design with 8 treatments and 4 replications. Data collected are SE cocoa populations, some problems of SE cocoa developments and social-economic of farmers. Plant observations include growth and productivity of cocoa. The data has been collected, tabulated described and analyzed by ANOVA and Duncan's Multiple Range Test (level 5%). SE cocoa plants have high jorget, small pod, small beans and flat. Productivity of side grafting cocoa is 4.21-4.36kg/tree/year. Response of farmers can receive bud grafting and side grafting technology for SE cocoa plant.

* **Corresponding Author:** Muhammad Basir Nappu ✉ basir@pertanian.go.id

Introduction

Cocoa development programing Indonesia are confront with various obstacles such as less of crop productivity, increasing population of old plants and poorly maintained crops, the presence of various pests or diseases, low seedling quality, undeveloped of downstream domestic industry, and the difficulty for farmers to get special funding for cocoa development program (Directorate General of Estate Crops, 2012).

Base on background of the above issues, in order to improve the productivity and quality of national cocoa, the National Cocoa Movement (NCM) program was established. This activity was initiated in 2009 with Rp. 302 billion and it was allocated for 11 regencyie. Luwu Utara, Luwu Timur, Luwu, Enrekang, Soppeng, Sidrap, Wajo, Soppeng, Bone, Bantaeng and Bulukumba. This program undertakes the rejuvenation, rehabilitation and intensification of cocoa development area of 48,200 ha consisting of 4,300 ha for rejuvenation, 20,900 ha for rehabilitation activities and 23,700 ha for intensification (Harian Fajar, *in* Limbongan *et al.*, 2012).

Lack of seedlings become obstacles in the cocoa plantation revitalization program, in 2010 the revitalization requires cocoa seeds up to 75 million seedlings to support the development of an area of 200,000 ha, but the availability of seedlings is only 57 million (Indonesian Plantation Research Institute, 2008). In the nature, T. cocoa multiplies via cross pollination. The seeds usually have many genetic backgrounds which may obtain crops that may be very different from each other ergonomically. In the other hand, reproductions by cloned graft materials tend to show bushy pattern which in a disadvantage. (Ramirez *et al.*, 2017)

SE technology can provide seedlings in large quantities, relatively faster available, and produce identical plant genetic with the parent and normally morphology. The somatic embryogenesis plant has a perfect crown, tap root, complete vigor, and is capable to producing faster and productive, and drought-tolerance (Winarsih *et al.*, 2002). During the NCM program, SE seedlings distribution data from 2009 to 2012 in South Sulawesi reached a total of 15,150,000 and recorded as follows 2009: 4.3 million seedlings; 2010: 3.55 million seedlings; 2011: 6.3 million seedlings, and 2012: 1.0 million seedlings (Anonymous, 2012).

The efficacy and success of SE seedlings production in the laboratory is quite widely reported. However, some research resulted in the weakness of SE seedlings in the field. Approximately 70-80% of farmers in Luwu and Luwu Utara consider cocoa plants from SE were less adaptive in the field (Limbongan *et al.*, 2011). Further study Limbongan *et al.* (2012) suggests that up to the third year, the percentage of cocoa plants from SE were decreasing. Center for Research and Natural Resource Development, Hasanuddin University, said that 74% of cocoa rejuvenation areas using SE seedlings in South Sulawesi and West Sulawesi are damaged, only 26% are found to be growth but in unhealthy conditions (Anonymous, 2012).

The government plan to evaluate the use of Somatik Embryogenesis (SE) cacao seedlings (Sinar Tani, 2014). The reason is that since three years of the National Movement of Cocoa Production and Quality Improvement NCM program, SE cocoa seedlings have not shown any increase in productivity. Through this study is expected to apply the technology of bud grafting and side grafting for somatic embryogenesis (SE) cocoa which has less of growth and yield.

Materials and methods

Time and Locations

The assessment was conducted in January 2015 to Desember 2016, in several regions which are the central of cocoa plantation in Bone and Luwu regency, South Sulawesi. In the two regency, the farmers received somatic embryogenesis (SE) cocoa seedling since the NCM program was launched, so these regions were founded SE rejuvenation assistance since 2009 to 2010.

Methods

This study used survey methods with interviewed 50 farmers from two regencies and a Randomized Block

Design with 8 treatments and 4 replications. The formulation of the treatment combinations:

G1C1 = Bud-grafting with Sulawesi-1 clone
G1C2 = Bud-grafting with Sulawesi-2 clone
G1C3 = Bud-grafting withmcC-01 clone
G1C4 = Bud-grafting withmcC-02 clone
G2C1 = Side-grafting with Sulawesi-1 clone
G2C2 = Side-grafting with Sulawesi-2 clone
G2C3 = Side-grafting withmcC-01 clone
G2C4 = Side-grafting withmcC-02 clone

The assessment materials include SE cocoa trees that were 6-7 years old that menaged relatively similar as rootstock and 4 locally superior clones as scion. Data colections from survey activity were SE cocoa populations, some problems of SE developments and social-economic of farmers. Plant observations include growth and productivity of cocoa, including: diameter of stem, bud length, number of bud, number of bud leaves, percentage of plan life, percentage of succeeded grafting, and operational costs of side grafting and bud grafting.

The collected data were tabulated, descripted, to analyze farmer feedback on vegetative technology on improved productivity and quality of cocoa. Furthermore, financial analysis to determine the amount of cost incurred for bud and side grafting treatment. Meanwhile, differences of the treatments analysed by Duncan's test at 5% level.

Stages of activities

Activities in the first year that had been undertaken to achieve the objectives of the activity were: consultation and coordination with relevant agencies, determination of location, initial data collection, and field experiment. Consultation and coordination were conducted on two locations of activities, Bone and Luwu regency. In consultation and coordination, the local government suggested the location of activities based on the cocoa NCM program area. Thus, the directions and suggestions from Local Government were followed up with determination of the assessment location and field experiment. The location of assestment in Bone Regency was Sungai Subur Farmer Group in Pattirobuli Village, Bengo District; while the location in Luwu Regency was Harapan Jaya Farmer Group, Pattedong Village, South Ponrang District. The treatments were to perform side-grafting techniques on SE cocoa plants with superior local clones ie: Sulawesi-1 (S-1), Sulawesi-2 (S-2), Masamba Cocoa Clone-01 (MCC-01), and Masamba Cocoa Clone 02 (MCC-02). In this activity a short interview were done with the farmers implementing the problems of cocoa plant that developing from somatic embryogenesis techniques. Cultivation, maintenance, observation and collect data from field experiment was done in the second year.

Results

Farmer's Characteristics

Cocoa farmers who had received cocoa rejuvenation with SE seedlings were sampled as cooperative farmers. A SE cocoa assistance for farmer cooperator in Bone Regency was iniciated in 2010 and Luwu Regency in 2009. The interviews with cooperative farmers are showed in Table 1.

Some farmers who already understand side grafting technology can improve the quality of cocoa crops and pods, in result the farmers applied side grafting on SE cocoa plants. Results of observations in the field showed that SE cocoa crops with high jorget and small pod that does not meet the expectations of farmers. SE cocoa plant that was grafted with S-2 clone in both areas are according to the expectation of farmers. However, field observations are only done on side grafts with other clones.

According to Table 1 that cocoa farmers in both Luwu and Bone regency belong to the productive age group. These conditions provide an overview the of farmers to adopt cocoa production technology. The experience of cocoa farmers is quite high with an average of 8.6 years and 13.75 years. The higher the farmers experience will provide the ability to manage the cocoa farming in the field. Family members of the farmers also include families who are less dependent or belonging to the family category prosperous. However this can be a problem in meeting the needs of farming activities.To solve this, hiring workers outside family (Limbongan and

Taufik, 2011). Land ownership for cocoa farming, Luwu farmers is wider than Bone farmers. The mastery of the land area is composed of the number of cacao plants grown on the land. Likewise, the acceptance of SE cacao seedling is more than for Luwu farmers.

Table 1. Identity and response of cocoa farmer for SE cocoa in South Sulawesi, 2015.

No	Description	Location	
		Bone Regency	Luwu Regency
1	Age (year)	41.0	43.8
2	Education (year)	13.75	8.6
3	Family member (person/fam)	1-5	2-5
4	Land area (ha)	0.98	1.95
5	Number of cocoa plants/farmer	850	1.800
6	Number of SE cocoa plants/farmer	438	750
7	Year of SE planting	2010	2009
8	Plant growth	Good	Jorget 1.5 m
9	Yields of cocoa pod	Many, small	Many, small
10	Yields of cocoa beans	Small and flat	Small and flat

Farmers who received cocoa seedlings from SE after planting for 6 years rated that the plants have optimum growth and have many pods. There is another type of *T. cocoa* cloning that utilizes totipotent cells from somatic tissue which is termed SE. These totipotent cells grow into embryo and then into plants.

Healthy planting materials can be obtained in abundance by this method. Variation of genetic background can also be minimized which is observable in better agronomic characteristics. However, there are several complaints submitted by farmers which are, high jorget, easy to uprooted, small pod and flat beans.

Plant Growth

Farmers, cooperators have received assistance through the National Movement of Cocoa Program by rejuvenation SE cocoa plants. But farmers' expectations say that plant growth and production are not optimal. So that, a technical assessment of side grafting and bud grafting with local superior cocoa clones was conducted. The results of the observations in Luwu Regency are showed in Table 2. Increasing the quality of cocoa plants and pods can be achieved by plant propagation through vegetative technology. The propagation for rejuvenation of plants obtained by bud grafting while rehabilitation on SE cocoa plant by side grafting, as well.

Table 2. Shoots growth of bud grafting and side grafting for 60 days in Luwu regency, 2015.

Treatments		Bud high(cm)	Trunk diameter (cm)	Number of branchs	Number of leaves
Simbols	Descriptions				
G1C1	Bud grafting S-1	$8{,}18^{d}$	$1{,}20^{c}$	$4{,}7^{c}$	$6{,}4^{d}$
G1C2	Bud grafting S-2	$8{,}43^{d}$	$1{,}07^{cd}$	$5{,}3^{bc}$	$6{,}1^{d}$
G1C3	Bud graftingmcC-01	$7{,}72^{d}$	$0{,}80^{e}$	$3{,}7^{d}$	$5{,}7^{e}$
G1C4	Bud graftingmcC-02	$8{,}15^{d}$	$0{,}95^{de}$	$4{,}8^{c}$	$6{,}2^{d}$
G2C1	Side grafting S-1	$17{,}45^{b}$	$1{,}95^{a}$	$6{,}4^{a}$	$8{,}1^{a}$
G2C2	Side grafting S-2	$18{,}92^{a}$	$1{,}97^{a}$	$6{,}5^{a}$	$7{,}9^{ab}$
G2C3	Side graftingmcC-01	$15{,}47^{c}$	$1{,}62^{b}$	$5{,}8^{b}$	$6{,}9^{c}$
G2C4	Side graftingmcC-02	$16{,}68^{bc}$	$1{,}90^{a}$	$6{,}3^{ab}$	$7{,}5^{b}$
DMRT (5%)		1,06	0,15	0,5	0,4

Note: The same letter in the same column is no different in the Duncan test of 5% level.

The high growth of cocoa side grafting plant with scions from S-2 gives a real difference to all good shoot and side grafting treatment with certified cocoa clones. While the growth of stem diameter on side grafting with S-1, S-2, andmcC-02 were not significantly different and had better growth but were significantly different from other treatments.

Leaf parameter is one of the indications to determine the quality of the plant growth. The observation that SE cocoa plant where side grafting with S-1 scion gives the best growth and differs significantly with other clones. In aggregate of several shoot growth parameters, stem diameter, number of branch and number of leaf giving the best valuation were SE cocoa plants very appropriate grafted with S-2, S-1 andmcC-02 clones.

Table 3. Shoots growth of bud grafting and side grafting for 60 days in Bone regency, 2015.

Treatments		Budhigh(cm)	Trunk diameter (cm)	Number of branchs	Number of leaves
Simbols	Descriptions				
G1C1	Bud grafting S-1	6.17^{d}	0.85^{b}	4.40^{d}	6.05^{d}
G1C2	Bud grafting S-2	6.42^{d}	0.77^{bc}	4.20^{de}	5.85^{d}
G1C3	Bud graftingmcC-01	5.72^{d}	0.70^{c}	3.75^{f}	5.17^{f}
G1C4	Bud graftingmcC-02	6.15^{d}	0.87^{b}	4.02^{e}	6.17^{d}
G2C1	Side grafting S-1	14.07^{b}	1.50^{a}	5.80^{a}	7.07^{b}
G2C2	Side grafting S-2	15.10^{a}	1.60^{a}	5.90^{a}	8.02^{a}
G2C3	Side graftingmcC-01	11.98^{c}	1.35^{b}	5.02^{c}	6.72^{c}
G2C4	Side graftingmcC-02	13.38^{b}	1.50^{a}	5.45^{b}	7.35^{b}
G1C5	DMRT (5%)	0.862	0.13	0.32	0.32

Note : The same letter in the same column is no different in the Duncan test of 5% level.

Shoots growth of bud grafting and side grafting of cocoa plants in Bone Regency used scions of S-1, S-2,mcC-01, andmcC-02 was showed in Table 3. Measurements on growth parameters of cocoa side-grafting treatment with S-2 scion gave the best increase and significantly different from other treatments. Meanwhile, stem diameter of side grafting treatment on SE cocoa with S-1, S-2, andmcC-02 clones gave the best increase and significantly different from other treatments. Similarly, increasing number of branches on side grafting with S-1 and S-2 clones gave the best increasing number of shoots, and significantly different with other treatments. The best leaf number increase was seen in side grafting treatment with Sulawesi-02 clone. Overall, the best treatment of cocoa quality improvement through bud grafting and side grafting in Bone Regency are bud grafting and side grafting treatment of SE cocoa with S-2 clone. This shows that SE cocoa plant in Bone Regency in terms of vegetative growth parameter is better compatibility by side grafting with S-2 clone. However, to see the results of side grafting compatibility on SE cocoa to the results of further research needs to be done. Quality improvement technology of cocoa either through rejuvenation with seedlings or rehabilitation of SE cocoa plant with side grafting both in Bone and Luwu regency give different result. In general side grafting treatment results in a better increase in all treatments.

Table 4. Growth average and productivity of 2-3 years side grafting by S-02 clones, in Luwu and Bone Regency, 2016.

Observation Component	Luwu Regency	Bone Regency
2 years old graft		
Shoots high (cm)	285	226
Number of branches	6	4
Number of pods per year	50 – 60	45 – 60
Dry seed production (kg/plant / per year)	2,56	2,21
3 years old graft		
Shoots high (cm)	315	340
Number of branches	5	6
Number of pods per year	95 – 125	80-120
Dry seed production (kg/plant / per year)	4,36	4,21

Some farmers who already understand side grafting technology can improve the plant growth and quality of pods, so the farmers do side grafting on SE cocoa plants. Results of observations in the field shows that SE cocoa plant with high growth of jorget and less number of poddoes not meet the expectations of farmers. In both area, the farmer hoped that growth and productivity of SE cocoa plant will be increase after grafting with S-2 clone.

Cost Analysis of Bud Grafting and Side Grafting

Improving the quality of cocoa plants and beans productivity can be done with rejuvenation oldplant by new seedlings and by side grafting technology. Both technologies require different costs. The analysis of technological cost of improving the quality of crops and cocoa beans was showed in Table 5.

Table 5. Cost analysis of bud grafting and side grafting (Rp. per ha year), 2016.

Materials and activity	Bud grafting	Sidegrafting
Materials		
1. Polybags	200,000	x
2. Manure	1,500,000	1,000,000
3. NPK fertilizer	115,000	230,000
4. Pesticides	45,000	45,000
5. Scissors, grafting knife, plastic, strap etc.	250,000	250,000
6. Entris	150,000	150,000
Wages		
1. Polybags charging	50,000	x
2.Maintenance of seedlings nursery	450,000	x
3. Bud grafting	450,000	x
4. Planting	150,000	x
5. Side grafting	x	1,500,000
6. Making planting hole	5,000,000	x
7. Planting	5,000,000	x
8. Maintenance of plants in the field (watering, fertilizing, weeding, pest /disease control, pruning etc.)	300,000	1,500,000
Total Cost(Rp)	13,660,000	4,675,000

Note : 1 US $ = Rp. 13,400.

Table 5 shows that improving the quality of plant growth and bean production by rejuvenating and rehabilitation, requires more cost for bud grafting compared to side-grafting technologies. The cost of rejuvenating cocoa plants with bud grafting reached Rp. 13.660.000 /ha in the first year. The highest cost component is making planting hole and planting reach Rp. 10,000,000 /ha or 73.21% of the total cost. Provision of planting materials can be done by purchasing the cocoa seed breeders or provide their own.While improving the quality of crops and cocoa beans with side-grafting technology requires less cost. This is because the existing plants rehabilitated only by side grafting and not new planting. The largest cost components are side-grafting and maintenance costs. However, if this sampling activity is done by the farmers themselves, then this cost can be used as saving or implisit cost.

Discussion

Cocoa farmers at the research sites are generally grown up and have a good ability to develop cocoa cultivation technology. To cultivate the land and maintain optimal crops, farmers still need labor from outside the family. After 6 years of cultivating SE cocoa plants, farmers generally find good cocoa plant growth but high jorget reaches 1.5 meters so plant easy to fall, pod many but little size. Different from the statement Garcia *et all.*, 2018 that there is another type of *T. cocoa* cloning that utilizes totipotent cells from somatic tissue which is termed SE. These totipotent cells grow into embryo and then into plants. Healthy planting materials can be obtained in abundance by this method. Variation of genetic background can also be minimized which is observable in better agronomic characteristics.

Likewise, the beans produced are small and flat. The weakness encourages farmers to look for technological alternatives that can be applied to improve the growth and productivity of SE cocoa plants they planted since 6 years ago.

One of the evaluation materials is the cause of the delayed crops, cocoa from SE seedling. Until now there is no reports of cocoa crops SE significantly increase yield (Sinar Tani, 2014). Limbongan *et al.* (2014) stated that the cocoa crops of SE reproduction in Gernas in 2009 and 2010 the quality of growth and production is very low. The above description shows the condition of Gernas cocoa program in 2009 and 2010 reached 7,850 ha or 7,850,000 plants (Anonymous, 2012). Therefore, it is necessary to improve the productivity and quality of the plants in order to support increased production and quality.

Vegetative propagation through bud grafting and side grafting can be selected as an alternative technology to improve the growth and productivity of SE cacao plants.The level of farmers' ability to carry out bud grafting and side grafting techniques can be seen from the percentage of sucsesfull connections. According to Limbongan and Djufry (2013) the percentage of sucsesfullconnections that experienced farmers with experience of grafting 1-3 years ranges

53% to 74%. Several studies such as Harman *et al.* (1997), Limbongan (2011), Limbongan *et al.* (2012) had successed connections only 52% others such as Salim and Drajat 2008; USAID (2008); Limbongan, (2012[b]); Limbongan *et al.* (2014) that is 73-97%. The success rate of this connection is determined, among others, by the experience of the farmers, the type of clone used as the scion, and the technical implementation. According to USAID 2008, farmers only take 2 years to become a means of skills.

Growth of shoots both bud grafting and side grafting can be seen from the development of plant height, stem diameter, number of branches, and number of leaves. In general, the growth of side grafting is better than bud grafting in Luwu and Bone. The result of observation at 2 months of age at both sites showed that the side grafting result showed that the shoot height reached 18.9cm, stem diameter 1.9cm, the number of branches 6 pieces and the number of leaves 8 sheets while the bud grafting only reached the shoot height of 8.4cm, the stem diameter 1.2cm, the number of branches 5 pieces, and the number of leaves 6 sheets. This condition can occur because of the capability of nutrient supply for the growth of larger shoots on the rootstock of the adult plant side grafting, while the capability of rootstock of the bud grafting is smaller. Although there are differences at the beginning of growth but poding agealmost simultaneously and usually depends on type of the scion. The results of observations Limbongan *et al.,* 2010 showed the poding age ranged between 2-3 years. There is a tendency of shoot growth difference due to the difference of the upper trunk clone, where the growth of clone S-1 is better than the other clones. Clones S-1 and S-2 have been recommended by the government as the superior clones of Sulawesi (Limbongan, 2012[a]) and many other local clones that proved to have good potential to be the source of scion. (Limbongan *et al.*, 2013).

SE cocoa planted with clone S- 2 in year 2 can produce 45-60 pieces pods per tree per year. In year 3 it increased to 80-125 pieces pods per tree per year or equivalent to 4.2-4.3kg beans/tree/year, or equivalent to 2.9 t dried beans/ha/year. The yield of dry beans obtained from the research Limbongan *et al.,* 2010; Salim and Dradjat, 2008 reached 2.34 t /ha /year.

The productivity of SE cocoa after being grafted with superior clones is quite able to give hope and new spirit for farmers to improve their SE cocoa plants. With such productivity, it is not inferior to the production achieved from bud grafting like cocoa such as cacao bud grafting in Puerto Rico that can produce 2,170kg dried beans /ha /year (Irrizari and Goenaga, 2000)

The analysis of farming in the second year showed that bud grafting cost 3 times bigger than side grafting. Where high difference cost component at the bud grafting are making the plant hole and planting, while side grafting and plant maintenance are the high cost on side grafting. The cost of bud grafting implementation is also greater than the cost of execution of side grafting. However, it does not mean that farmers prefer side grafting than bud grafting, but both can be an alternative choice to improve the growth and productivity of SE cocoa. According to Anita and Susilo (2012) side grafting technology is used for adult crops that are unproductive because they are old or because they come from unproductive clones such as SE cocoa. Meanwhile, the propagation multiplication technology of bud grafting is the most widely applied in vegetative propagation technology of cocoa farmers (Limbongan *et al.,* 2013). Cocoa seedlings produced through bud grafting technology can be used both for new openings and embroidery on SE cocoa cultivation.

Conclusions

The technique of bud grafting and side grafting cocoa with local clones as an effort of rejuvenation is acceptable and according to expectations of farmers. Cocoa plants from SE that have high jorget, small pod and flat can be improved through bud grafting and side grafting techniques with local cocoa superior clones. Farmers can accept the technology of bud grafting and side grafting with paying attention to SE cocoa plant that has been grafted with cocoa clone S-2. Based on the above descriptions and conclusions, further research needs to be done on the cultivated cocoa bud grafting and side grafting, as the cocoa plant is included in the annual crop group.

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